United States Department of Agriculture-Agricultural Research Service, Morris, MN and Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, MN, USA

Ionic Ratios and Crop Performance: II. Effects of Interactions amongst Vanadium, Phosphorus, Magnesium and Calcium on Soybean Yield

A. Olness, D. Palmquist and J. Rinke

Authors' addresses: Dr A. Olness (corresponding author; e-mail: olness@morris.ars.usda.gov) and Ms J. Rinke, United States Department of Agriculture-Agricultural Research Service-MWA (USDA-ARS-MWA), N. Central Soil Conservation Research Laboratory, 803 Iowa Avenue, Morris, MN 56267; Dr D. Palmquist, USDA-ARS-MWA, Office of the Area Director, RM 3026, 1815 N University Street, Peoria, IL 61604, USA

Abstract

Because vanadium (V) is easily reduced to a cationic form within plant cells, data from resin-extraction of soil were analysed for evidence of interactions between V and the resin-extractable concentrations of magnesium (Mg) and calcium (Ca) on soybean seed yield. Three varieties, 9091, 9061 and 704, were grown over a 3-year period in a corn–soybean–wheat rotation. Surface soil samples (0–15 cm) were extracted with ion-exchange resins, extracts were analysed by inductively coupled plasma methods (ICP), and the results were regressed against seed yield using SAS PROC STEPWISE analysis using forward selection, backward elimination and maximum R^2 routines. The seed yield of each variety showed a correlation with a unique set of resin-extractable concentrations of V, phosphorus (P), Mg and Ca, and the V:(V + P), Mg:(Mg + Ca), Mg:(Mg + 1000 V) and Ca:(Ca + 1000 V) ratios. Variety 9091 was most sensitive to the Mg:(Mg + Ca) ratio. Variety 9061 was most sensitive to extractable V and to the V:(V + P) ratio. Variety 704 was sensitive to extractable P, V and Ca and the Mg:(Mg + 1000 V) ratio. For variety 9091, Mg fertilization (not currently practised) may be an economical practice, whereas P fertilization of 704 may not be economical. Each regression technique varied slightly in identification of important factors in seed yield. Concentrations and ratios of resin-extractable elements in soil provide insights into optimal genotype selection and possible management alternatives for a given soil.

Key words: calcium — *Glycine max* — magnesium — soybean — phosphorus — resin-extraction — vanadium

Introduction

Earlier we noted the effect of the resin-extractable ratios of V:(V + P) in soil on soybean seed yield and the interaction with variety (Olness et al. 2000). Unlike phosphorus (P), vanadium (V) is rather easily reduced and, as a consequence, it is converted to the cationic vanadyl form (+4 valence) within the cell (Liochev and Fridovich 1987, Ding et al. 1994).

As a divalent cation, we might expect interactions within the plant cell between VO^{2+} and Ca^{2+}, Mg^{2+} and other cations. Both Ca^{2+} and Mg^{2+} are required in rather large amounts by plants for optimal seed yield. Magnesium (Mg), in particular, plays important roles in ATP formation, chlorophyll formation, protein synthesis, cellular pH control, and enzyme activation (see citations in Marschner 1986). Vanadium is a potent enzyme inhibitor and has been used to characterize enzymes catalysing phosphate transfer, in which Mg^{2+} plays a major role. While large amounts of calcium (Ca) are contained in the middle lamella of the cell wall, where it performs a structural function, it is also important in membrane stability, root extension and enzymatic regulation.

With a hydrated ionic radius of 0.428 nm, Mg^{2+} is similar to Ca^{2+} (0.412 nm) in physical dimensions. Does the ratio of these elements in soil have measurable effects on soybean seed yield and do the relative concentrations of Mg^{2+}, Ca^{2+} and V affect seed yield? To address these questions, we reviewed the data obtained in an earlier study.

Methods and Materials

The field site consisted of 3.24 ha measuring 108 m by 300 m on the Barnes–Aasted Association's Swan Lake Research Farm located 24 km NNE of Morris, MN, USA.

Table 1: Descriptive statistics of the resin-extractable elements and ratios

Statistic	nmol g⁻¹		μmol g⁻¹		mol mol⁻¹			
	P	V	Ca	Mg	V: (V + P)	Mg: (Mg + Ca)	Ca: (Ca + 1000 V)	Mg: (Mg + 1000 V)
Average	443	1.65	8.65	4.44	0.022	0.394	0.815	0.394
Minimum	nd[1]	nd	0.152	0.166	0.000	0.092	0.031	0.034
Maximum	3010	18.3	28.3	24.2	0.405	0.725	1.00	1.00
Median	221	0.757	5.26	3.43	0.003	0.414	0.953	0.887

[1]nd = not detectable.

Soil taxonomic units were delineated by a detailed soil survey conducted by the local NRCS Soil Survey (see Olness et al. 2000). Soils at the site were Barnes loam (fine-loamy, mixed Udic Haploboroll), Buse loam (fine-loamy, mixed, Udorthentic Haploboroll), Hamerly clay loam (fine-loamy, frigid, Aeric Calciaquoll) and Parnell clay loam (fine, montmorillonitic, frigid typic Argiaquoll). Less than 0.1 % of the area was Svea sandy loam (fine-loamy, mixed, Pachic Udic Haploboroll; USDA 1972) and these plots were omitted from the study.

The site was divided into three equal portions and planted to soybean, maize and wheat, and crops were rotated annually. Each crop area was further subdivided into 360 plots measuring 3 m by 10 m and planted to soybean or maize in 1995 and soybean, wheat and maize in rotation in 1996 and 1997. Soybean varieties are designated 704, 9091 and 9061. Each plot consisted of four rows of soybean planted at a rate of 65 seeds m⁻² at 0.75-m row spacing. Each variety was planted continuously (that is, without subplot borders) over the 300-m length of the field. Soybean seed yields were taken from the central 8 m of the two central rows of each plot with a plot combine and samples were withdrawn for further characterization.

Soil samples were taken from alternate plots by compositing five core samples (0–60 cm) taken from the centre two rows. The core samples were segmented into 15-cm increments. The soils were dried at about 60°C and ground for chemical analyses. Extractions were conducted using extractors described by Olness et al. (1989) and the procedure described by Olness and Rinke (1994), with the exception that the extracting solution contained 20 % (v/v) methanol to inhibit microbial activity. In 1995, extractions were carried out over a 5-day period with a brief manual shaking twice daily. The procedure was changed to include slow continuous shaking in 1996 (rapid mechanical shaking is avoided to preclude abrasion of the membrane). After elution, extracts were submitted to the Analytical Research Laboratory of the University of Minnesota, St. Paul for ICP-AES (inductively coupled plasma – atomic emission spectroscopy) analysis. Statistical characteristics of the data set are given in Table 1.

Because numbers of plots within a soil mapping unit varied and the soil characteristics are changing continuously, SAS PROC STEPWISE with the forward selection, backward elimination and maximum R^2 options was used to analyse yield and soil chemical data (Ray 1982). Yields from a few plots were noticeably affected by excess water, which reduced plant population densities; therefore, seed yields from plots with < 2000 kg ha⁻¹ were also excluded. This decision removed about 5–8 yield values (< 2.5 %) each year from the data set; all came from Hamerly clay loam and Parnell clay loam mapping units.

Results and Discussion

A preliminary examination of the data might suggest that climate had an influence on seed yield (Table 2). Seed yields in 1996 tended to be slightly lower than those in 1995 or 1997. However, no variety was dominant every year, and this suggests more subtle factors were affecting plant response. For example, in 1995 variety 9091 clearly produced greater yields than variety 9061, but in 1997 variety 9061 clearly produced greater yields than 9091. Note that statistical analyses are only for plots from which soil samples were extracted with resins and not all plots as reported in Olness et al. (2000). A review of the P and V data from 1997 (not published) showed yields were consistent with our earlier report (Olness et al. 2000).

Variety 9061

Our initial results showed that variety 9061 is sensitive to the V:(V + P) ratio (Olness et al.

Table 2: Annual soybean seed yields

Year	Yield[1] (megagrams ha⁻¹)		
	Soybean variety		
	704	9061	9091
1995	—	3.05 b β	3.36 a α
1996	2.90 b β	2.88 c β	3.15 b α
1997	3.10 a β	3.32 a α	3.16 b β

[1] Seed yields within a column followed by the same Roman letter were not measurably different ($P < 0.05$). Seed yields within a row followed by the same Greek letter were not measurably different ($P < 0.05$).

2000), and all regression techniques identify this as an important factor (of the eight) in determination of yield (Table 3). It appears that V may have a negative effect on seed yield that is independent of P. The V:(V + P) ratio effect is expected if both V and P are being transported across the plasma lemma by the same suite of proteins. The V:(V + P) ratio remained a strong factor in two of the three analyses. This ratio effect is consistent with Bowman's (1983) observation of competitive transport across the plasma lemma of these two elements by a set of proteins.

Both forward selection and maximum R^2 techniques indicated a negative effect on seed yield of the concentration of resin-extractable Mg. This is mildly surprising because VO^{2+} within the plant cell is a candidate for interference with the functions of Mg, and a positive relationship was expected. However, the north-western Cornbelt region is relatively rich in both Ca and Mg and the effect may be a response to, or indication of, the fact that 9061 is nearing its environmental limit (soil and rainfall) with respect to soluble salts.

The remaining variables either contributed little to explaining the variance in seed yield or were important in only one of the techniques. While an interaction of Ca and/or Mg with V might reasonably be anticipated, no evidence was found. Vanadium is poorly translocated within many plants and the effects could easily be confined to the roots.

The intercept is a relative measure of the site-specific yield potential, and for 9061, the values averaged about 3.0 megagrams ha^{-1} with a standard deviation of about 0.22 megagrams ha^{-1}. Of the factors identified in this analysis, most had a negative coefficient; so the intercept appeared to be a near-maximal yield potential. The correlation coefficient of about 0.304 showed that most of the variability in seed yield was due to other factors, and certainly temperature and water rank highly among them.

Variety 704

Variety 704 (excluded from our earlier report because it had been grown for only 1 year) appeared to be the most nutritionally sensitive variety of the three. Variety 704 showed a distinct increase in seed yield as resin-extractable P increased, but the effect was small and overwhelmed by the loss of yield potential from the V:(V + P) ratio effect (data not shown). In its sensitivity to V, 704 resembled variety 9061 (Table 3). This

Table 3: Statistical summary of eight variable coefficients affecting soybean yield at the Barnes-Aasted Research Farm

	Regression analysis technique[1]								
	Forward selection			Backward elimination			Maximum R^2		
REx[2] variable	9091	704	9061	9091	704	9061	9091	704	9061
Intercept (Mg ha^{-1})	2.32	3.69	2.81	2.71	3.69	3.25	2.30	3.81	2.97
V	ns[3]	**-1.63×10^{-1}**	**-4.06×10^{-2}**	ns	**-1.63×10^{-1}**	**-4.37×10^{-2}**	4.09×10^{-3}	**-1.54×10^{-1}**	**-4.00×10^{-2}**
P	ns	**8.82×10^{-4}**	ns	ns	**8.82×10^{-4}**	ns	-2.41×10^{-6}	**9.35×10^{-4}**	4.15×10^{-5}
Mg	-2.87×10^{-2}	ns	-4.19×10^{-2}	ns	ns	ns	-2.95×10^{-2}	-5.95×10^{-3}	**-3.82×10^{-2}**
Ca	1.46×10^{-2}	**9.31×10^{-3}**	**2.47×10^{-2}**	ns	**9.31×10^{-3}**	ns	1.46×10^{-2}	8.48×10^{-3}	2.03×10^{-2}
V:(V + P)	-1.88	**-1.09**	**-3.67**	ns	**-1.09**	**-4.07**	-1.95	**-1.09**	**-3.24**
Mg:(Mg + Ca)	**2.09**	ns	1.02	**1.28**	ns	ns	**2.09**	-3.03×10^{-1}	6.14×10^{-1}
Mg:(Mg + 1000 V)	-0.94	-8.81×10^{-1}	ns	ns	-8.81×10^{-1}	ns	9.36×10^{-1}	-6.73×10^{-1}	4.21×10^{-1}
Ca:(Ca + 1000 V)	1.02	ns	ns	ns	ns	ns	1.04	-2.06×10^{-1}	-3.95×10^{-1}
R^2	0.265	0.485	0.317	0.215	0.485	0.276	0.265	0.490	0.320

[1] Bold-faced coefficients are the most important factors in terms of regression analysis probabilities (P < 0.10).
[2] All variables are given as resin-extractable concentrations in molar units (micro or nano) g^{-1} soil.
[3] ns = non-significant; that is, the statistical technique omits these variables within a variety and considers them non-significant factors in explaining the variance in seed yield.

sensitivity to P suggests that 704 was less efficient in P acquisition than the other two varieties. Unfortunately, the coefficient of P, while highly significant, was rather small and P management on this site may provide only modest improvements in yields, with the possible exception of banded applications of fertilizer P. Certainly, conventional soil testing would not suggest a strong response to added P (Olness et al. 2000).

Unlike 9061, 704 showed virtually no sensitivity to either Mg or the Mg:(Mg + Ca) ratio. Its rather pronounced sensitivity to a Mg:(Mg + 1000 V) ratio effect suggests an interference with a function(s) of Mg within the plant. The fact that the V component of this ratio is multiplied by a factor of 1000 hints at the extreme sensitivity of the plant and the Mg function(s) to the presence of V.

This variety had the largest average intercept yield potential, 3.73 ± 0.07 megagrams ha^{-1}. But, with the exception of P, most of the factors had negative coefficients so the intercept values appeared to be a near-maximal yield potential. This variety seems well suited to P-rich but V-poor environments. The multiple correlation coefficient was about 0.487; so, more of the variance in yield was explained by these factors and their complex interactions for 704 than for the other varieties.

Variety 9091

The main sensitivity of variety 9091 was to the resin-extractable Mg:(Mg + Ca) ratio (Fig. 1 and Table 3). The seed yield ranged from about 2.9 to 3.6 megagrams ha^{-1} as the Mg:(Mg + Ca) ratio changed from 0.16 to 0.62; this is about a 24 % change in yield. The importance of the Mg:(Mg + Ca) ratio was so great that Mg may be an economical fertilizer, even though these soils are relatively rich in Mg (unpublished greenhouse studies support this speculation). Like 9061, the yields of variety 9091 showed a modest and negative sensitivity to resin-extractable Mg. Thus, 9091 may also be nearing the limit of its ability to tolerate soluble salts in this environment. This variety would seem well suited to an environment relatively rich in available Mg.

Unlike the other varieties, 9091 showed little sensitivity to V or P. This lack of sensitivity to V demonstrates that breeding can produce varieties suited to a specific environment. The intercept or site-specific yield potential of this variety was the smallest of the three studied at 2.44 ± 0.23 megagrams ha^{-1}. Unlike varieties 704 and 9061, the
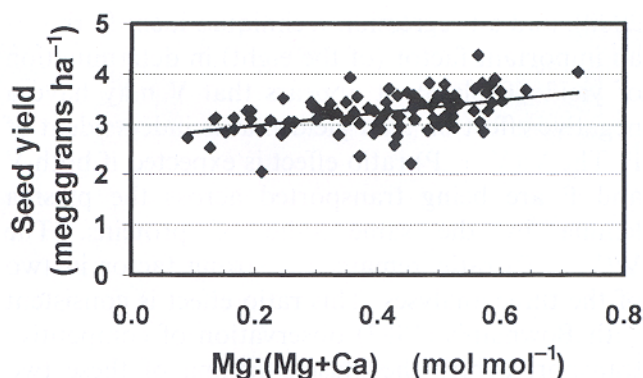

Fig. 1: The effect of the resin-extractable Mg:(Mg + Ca) ratio on seed yield of soybean variety 9091. The solid line linear model is: yield = 2.71 + 1.28 [Mg:(Mg + Ca)]; $R^2 = 0.215$

Mg:(Mg + Ca) ratio had a large positive effect on the yield of variety 9091, so much so that seed yields of variety 9091 were competitive with the other varieties. The R^2 values, about 0.215, showed that these resin-extractable components were satisfying a small but very important portion of the variance in seed yields and that most of this variance was due to the Mg:(Mg + Ca) ratio.

Speculation on sensitivities of varieties

Analyses of the data show that each variety is uniquely sensitive to a different set of nutrient supplies in the same environment. We have no ready explanation for the varied sensitivities of these soybean varieties to different resin-extractable chemistries. Each variety produces rather well in this region. We can, however, speculate that much of the difference may be explained by the suite of transport proteins within the plasmalemma and interactions with mycorrhizal fungi and other rhizosphere organisms. For example, the sensitivity of 9061 to the V:(V + P) ratio could arise from an inability of the transport proteins within the cell membrane to distinguish between V and P. In such a case, transport of V would come at a cost of P transport in addition to any internal interactions with other ions and the well-known inhibition of ATPases (Cantley et al. 1977).

Root growth for all three varieties seems enhanced by Mg in growth chamber experiments (data not shown). The near-exclusive sensitivity of 9091 to the Mg:(Mg + Ca) ratio may be due to an inability to discriminate between the two elements or may reflect the needs of its symbiont. Mycorrhizal fungi may need greater relative concentrations

of Mg, because the absence of vascular tissues should reduce the relative need for Ca in the fungi. Adverse effects of resin-extractable concentrations of Ca on internal enzymes within the plant seem poorly supported in variety 9091; the coefficient of Ca was either non-significant or positive and small.

Plant colonization by mycorrhizal fungi seems linked to P starvation-induced physiology changes in some plants (McArthur and Knowles 1992). The interaction between V and P (Olness et al. 2000) then should lead to greater mycorrhizal colonizations when the V:(V + P) ratio is relatively large. The mitigating Mg:(Mg + Ca) effect on both plants and fungi would permit plants to survive in soil environments with seemingly toxic levels of V.

Each regression technique provided a slightly different array of significant factors. While the relative significance of the factors varied between techniques, the indicated coefficients for each factor showed rather small variances. The most important factors were consistently identified by all regressing techniques. The regressions, alone, do not prove a relationship. However, the results are reasonably consistent with the literature and they provide an estimate of their importance.

The apparent climatic effect on seed yield is actually well explained by the changing chemical character of the soil in the three subdivisions of the field. Many of the changes in soil chemical nature were very subtle, but when they are considered it becomes clear that modest differences in climate between years (data not presented) had little effect on seed yield.

Conclusion

Varieties of soybean are uniquely sensitive to resin-extractable concentrations of nutrients from soil. Evaluation of yield and resin-extractable chemistry data can identify potentially profitable management programmes that maximize economical returns for each variety. The process of evaluation of the soil by variety interactions provides a basis for selection of the genotype and fertility management programme best suited to a particular soil. Resin-extraction of soils provides a suite of elements, and their relative concentrations can serve as a guide to plant selection for a specific environment. Developing plants suited to a soil may be much more economical than modifying soil to obtain optimal seed, forage, or fibre yield.

The observation that Mg is a probable profitable management factor in a Mg-rich environment shows the importance of determining the 'available concentrations' by use of an appropriate test. More importantly, complex interactions for both P and Mg show that simple evaluations of fertility components can miss important economic effects. It is also clear that varieties can be developed that are rather insensitive to the presence of levels of V (or other elements) capable of depressing seed yield in many crops.

Zusammenfassung

Ionenverhältnisse und Kulturpflanzenleistung: II. Interaktionen zwischen V, P, Mg und Ca bei Sojabohnen

Da Vanadium (V) innerhalb von Pflanzenzellen sehr leicht auf eine Kationform reduziert werden kann, wurden die Daten der Harz-Extraktion aus der Erde auf Hinweise der Interaktion zwischen V mit der Harz-Extraktionsfähigen Konzentration von Mg und Ca auf den Ertrag der Sojabohnensamen analysiert. Drei Varianten, 9091, 9061 und 704 wurden über einen Zeitraum von drei Jahren, in einer Korn–Sojabohne–Weizen Rotation, angebaut. Proben der Oberfläche des Erdbodens (0–15 cm) wurden mit Ionenaustausch Harzen entnommen, Extrakte wurden durch ICP analysiert und die Resultate wurden gegen Samenerträge verglichen unter Verwendung von SAS PROC STEPWISE Analyse mit Vorwärtsauswahl, Rückwärtselemenierung und maximal R^2 Prozeduren. Samenerträge von jeder Variation zeigten Korrelationen mit einer einzigartigen Gruppe von Harz-Extraktionsfähigen Konzentration von V, Mg, Ca und V:(V + P), Mg:(Mg + Ca), Mg:(Mg + 1000 V), Ca:(Ca + 1000 V) Verhältnissen. Variante 9091 war sehr sensitiv zu dem Mg:(Mg + Ca) Verhältnis. Variante 9061 war am meisten sensitiv zu dem extraktionsfähigen V und zu dem V:(V + P) Verhältnis. Variante 704 war sensitiv zu dem extraktionsfähigen P, V, Ca und dem Mg:(Mg + 1000 V) Verhältnis. Für Variante 9091 kann Mg Düngung (zur Zeit nicht praktiziert) ein wirtschaftliches Verfahren sein; andererseits kann P Düngung von 704 fehlschlagen bei der Erzielung von wirtschaftlichen Erträgen. Jede Regressionstechnik variiert leicht in der Identifizierung von wichtigen Faktoren in Samenerträgen. Konzentrationen und Verhältnisse von Harz-Extraktionelementen im Erdboden bieten Einsicht in optimale Genotypusauswahl und mögliche Managementalternativen für einen bestimmten Erdboden.

Acknowledgements

Erickson and Dan Swenson for assistance in sample collection and processing. Mention of a trademark name, proprietary product, or vendor does not constitute a guarantee of the product by the USDA and does not imply its approval to the exclusion of other suitable products or vendors.

References

Bowman, B. J., 1983: Vanadate uptake in *Neurospora crassa* occurs via phosphate transport system II. J. Bacteriol. **153**, 286—291.

Cantley, L. C., L. Josephson Jr, R. Warner, M. Yanagisawa, C. Lechene, and G. Guidotti, 1977: Vanadate is a potent (Na, K)-ATPase inhibitor found in ATP derived from muscle. Biol. Chem. **252**, 7421—7423.

Ding, M., P. M. Gannett, Y. Rojanasakul, K. Liu, and X. Shi, 1994: One-electron reduction of vanadate by ascorbate and related free radical generation at physiological pH. J. Inorg. Biochem. **55**, 101—112.

Liochev, S., and I. Fridovich, 1987: The oxidation of NADH by tetravalent vanadium. Arch. Biochem. Biophys. **255**, 274—278.

McArthur, D. A. J., and N. R. Knowles, 1992: Resistance responses of potato to VA-mycorrhizal fungi under varying abiotic phosphorus levels. Plant Physiol. **100**, 341—351.

Marschner, H., 1986: Functions of mineral nutrients: macronutrients. In: Mineral Nutrition in Higher Plants, pp. 195—267. Academic Press, Orlando, FL.

Olness, A., and J. L. Rinke, 1994: Resin-based extractor for multiple-element determination of availability in soils. Can. J. Soil Sci. **74**, 461—463.

Olness, A., C. Dahlem, and L. F. Bohlman, 1989: Isopotential Available Ion Extractor. Patent 4 816 161. U.S. Department of Commerce Patent Trademark Office, USGPO, Washington, DC.

Olness, A., T. Nelsen, J. Rinke, and W. B. Voorhees, 2000: Ionic ratios and crop performance. I. Vanadate and phosphate on soybean. J. Agron. Crop Sci. **185**, 145—152.

Ray, A. A. (ed.), 1982: SAS User's Guide: Statistics. Stat. Analy. Sys. Inst., Cary, NC.

USDA, Soil Conservation Service, 1972: Soil Series of the United States, Puerto Rico and the Virgin Islands: Their Taxonomic Classification. Supplement to Agriculture Handbook No. 436, Soil Taxonomy, A Basic System of Soil Classification for Making and Interpreting Soil Surveys. USGPO-0-465-739. US Department of Agriculture, Washington, DC.